Biological Control of Indianmeal Moth (Lepidoptera: Pyralidae) on Finished Stored Products Using Egg and Larval Parasitoids

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ABSTRACT Biological control using hymenopteran parasitoids presents an attractive alternative to insecticides for reducing infestations and damage from the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), in retail and warehouse environments. We examined the potential for using combinations of the egg parasitoid *Trichogramma deion* Riley (Hymenoptera: Trichogrammatidae), and the larval parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) for preventing infestations of *P. interpunctella* in coarse-ground cornmeal as well as the influence of packaging on parasitoid effectiveness. Treatments included one or both parasitoids and either cornmeal infested with *P. interpunctella* eggs or eggs deposited on the surface of plastic bags containing cornmeal. *H. hebetor* had a significant impact on the number of live *P. interpunctella*, suppressing populations by ≈71% in both unbagged and bagged cornmeal. In contrast, *T. deion* did not suppress *P. interpunctella* in unbagged cornmeal. However, when released on bagged cornmeal, *T. deion* significantly increased the level of pest suppression (87%) over bagging alone (15%). When *H. hebetor* was added to bagged cornmeal, there was a significant reduction of live *P. interpunctella* compared with the control (70.6%), with a further reduction observed when *T. deion* was added (96.7%). These findings suggest that, in most situations, a combined release of both *T. deion* and *H. hebetor* would have the greatest impact; because even though packaging may protect most of the stored products, there are usually areas in the storage landscape where poor sanitation is present.

KEY WORDS *Trichogramma*, *Habrobracon*, augmentation, packaging

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is an important and widespread pest of stored products in North America. It is capable of infesting a broad range of products, including raw and processed cereal products, animal feeds, dried fruit, nuts, and garlic (Cox and Bell 1991, Perez-Mendoza and Agulera-Peña 2004). In warehouses and retail stores, this insect infests finished products, which may lead to customer complaints. Furthermore, webbing created by larval *P. interpunctella* can complicate sanitation in processing facilities.

Management of *P. interpunctella* in warehouses and retail stores traditionally has depended on the application of insecticidal fogs, fumigants, and sprays (Cox and Bell 1991). However, new insecticide regulations resulting from the Food Quality and Protection Act as well as consumer concern over pesticide residues have limited the availability of insecticides for use in stored products (Arthur and Rogers 2003). Insect-resistant packaging and regular sanitation are also important management tactics. However, because damaged packages and spillage are common, these sources of pest infestation persist. A unique challenge for pest management on finished stored products is that, unlike crops, there is no potential for regrowth or recovery. Furthermore, infestation of a product with a single insect is sufficient to cause the loss of that product because of customer complaints, loss of future business, and even lawsuits (Subramanyam et al. 2001). Thus, products in warehouses and retail stores have a very low economic threshold.

Despite the low pest tolerance, biological control of *P. interpunctella* by using hymenopteran parasitoids provides one possible alternative pest management tactic. A variety of parasitoid species have been studied, including egg parasitoids in the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) as well as the larval parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae). *Trichogramma* spp. have been explored as potential natural enemies for a variety of stored product moths in bulk peanut storage (Brower 1988), bulk wheat storage (Schöller et al. 1994, 1996), and bakeries (Prozell and Schöller 1998, Steidle et al. 2001). *H. hebetor* also has been investigated as a biological control agent of stored product moths in bulk peanuts (Brower 1990), packaged products (Cline et al. 1984), and residual populations in grain spillage (Press et al. 1982).

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In other studies, the interactions between multiple natural enemies of *P. interpunctella* in raw commodities have been examined. Brower (1990) evaluated *H. hebetor* and *Trichogramma pretiosum* Riley in peanut warehouses and found that a combination of the two parasitoids provided the best biological control for this pest as well as for the almond moth, *Caudra cautella* (Walker) (Lepidoptera: Pyralidae). Keever et al. (1986) demonstrated that a combination of *H. hebetor* and the predator *Xylocorus flavipes* (Reuter) (Hemiptera: Anthocoridae) reduced trap captures of, and damage from, *P. interpunctella* and *C. cautella* in farmers stock peanuts in commercial warehouses.

The objective of our study was to determine whether a combination of the egg parasitoid *T. deion* and the larval parasitoid *H. hebetor* would be more effective than either parasitoid alone in suppressing *P. interpunctella* populations on both packaged and unpackaged cornmeal.

Materials and Methods

Insect Culture. Insects were reared at the USDA–ARS, Grain Marketing and Production Research Center, Manhattan, KS. Colonies of *T. deion*, *H. hebetor*, and *P. interpunctella* were maintained in walk-in growth chambers set at 26°C and 60% RH. *P. interpunctella* were reared on a standard cracked wheat, wheat shorts, glycerin, honey, and wheat germ diet (McGaughey and Beeman 1988). *T. deion* were reared on the UV-sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and *H. hebetor* were reared on the last instars of *E. kuehniella.* All parasitoids used in experiments were naive adult females. *T. deion* females were 4 to16 h postemergence, whereas *H. hebetor* females were 16–24 h postemergence. Both species were allowed access to honey and mates for 4 h before testing.

Experimental Design. The experiment was conducted in a walk-in growth chamber maintained at 26 ± 1°C and 60 ± 5% RH. A complete factorial design was used, consisting of two levels each (present or absent) of the parasitoids *T. deion* and *H. hebetor,* and packaging. There were five replications of the eight treatment combinations for a total of 40 experimental units. The bagged cornmeal was contained in 16.5- by 14.9-cm polyethylene resealable bags, each filled with 100 g of organically grown, coarse-ground cornmeal and perforated 16 times with a 0.5-mm-diameter push-pin to provide access for *P. interpunctella* neonates. The unbagged treatments contained the same amount and type of cornmeal. Treatments were set up in individual 1.89-liter Kroger brand plastic containers with lids that had a 4-cm-diameter hole covered with silkscreen cloth to allow for airflow.

One hundred freshly laid *P. interpunctella* eggs were evenly sprinkled directly onto the cornmeal in half of the containers (unbagged treatment) and onto the bagged cornmeal (bagged treatment) in the other half of the containers. In containers receiving *T. deion,* 10 females were released immediately after host eggs had been placed. In those treatments receiving *H. hebetor,* one adult female was added to each container on day 21, which corresponded to the presence of late instar hosts.

Containers were opened on day 45, ≈10 d after the appearance of the first *P. interpunctella* adults. The contents of each arena were individually sifted by using a series of sieves arranged in order of decreasing mesh openings (2.00, 1.41, 0.425, and 0.210 mm). The numbers of live and dead *P. interpunctella* larvae, pupae, and adults were recorded, along with pupal and adult *H. hebetor.* Each sample was sieved three times to ensure that all insects were collected.

Data Analyses. Data were analyzed as a one-way analysis of variance (ANOVA) with eight levels by using PROC GLM (SAS Institute 2000). A Fisher least significant difference (LSD) test was used to separate treatment effects. The combined total number of live and dead *P. interpunctella* (larval–adult stages) as well as the total number of live *P. interpunctella* were analyzed in individual ANOVA models because the presence of both live and dead stored-product pests can result in customer complaints.

Results

Live and Dead *P. interpunctella.* Treatment effects were highly significant for total dead and live *P. interpunctella* ($F = 23.6$; df = 7, 32; $P < 0.0001$). *T. deion* alone did not significantly reduce *P. interpunctella,* compared with the untreated control (Fig. 1). Packaging alone provided a small (15.6%), but significant, reduction of *P. interpunctella* compared with the control. The addition of *T. deion* to bagged cornmeal resulted in a very large (87.0%) reduction of *P. interpunctella* compared with the control (Fig. 1). Thus, suppression was increased by 71% by adding *T. deion.* On unbagged cornmeal, *H. hebetor* alone provided a significant reduction in the total pest population (46.4%), but adding the second parasitoid, *T. deion,* did not significantly increase suppression of *P. interpunctella* (Fig. 1). When *H. hebetor* was released on bagged cornmeal, there was a significant reduction in *P. interpunctella* (42.4%), compared with bagged cornmeal alone (15.6%) (Fig. 1). However, when both parasitoid species were released on bagged cornmeal, pest populations (total live and dead) were not significantly different from treatments where only *T. deion* was released on bagged cornmeal (Fig. 1).

Live *P. interpunctella.* Treatment effects were also highly significant for total live *P. interpunctella* ($F = 40.96$; df = 7, 32; $P < 0.0001$). Neither *T. deion* alone nor the bagged cornmeal alone treatments resulted in a reduction of live *P. interpunctella* compared with the control (Fig. 2). However, when *T. deion* were added in combination with bagged cornmeal, there was a very large reduction (87.1%) of live *P. interpunctella* compared with the control (Fig. 2). On unbagged cornmeal, *H. hebetor* reduced the number of live *P. interpunctella* compared with the control (72.4%). Although the addition of *T. deion* further reduced the number of live *P. interpunctella* (86.8%), the difference between parasitoid treatments was not signifi-

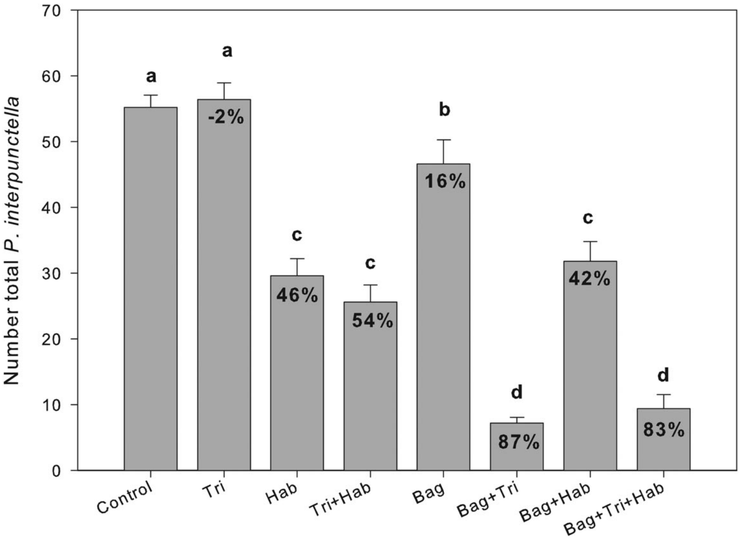

Fig. 1. Mean ± SEM total (live + dead) number of *P. interpunctella*. Percentage of reductions compared with control are shown inside bars. Control, without bag, without *T. deion*, without *H. hebetor*; Tri, without bag, with *T. deion*, without *H. hebetor*; Hab, without bag, without *T. deion*, with *H. hebetor*; Tri + Hab, without bag, with *T. deion*, with *H. hebetor*; Bag, with bag, without *T. deion*, without *H. hebetor*; Bag + Tri, with bag, with *T. deion*, without *H. hebetor*; Bag + Hab, with bag, without *T. deion*, with *H. hebetor*; and Bag + Tri + Hab, with bag, with *T. deion*, with *H. hebetor*. Different letters indicate statistically significant differences (Fisher LSD; $P = 0.05$).

cant. When *H. hebetor* was added to bagged cornmeal, there was a significant reduction of live *P. interpunctella*, compared with the control (70.6%), with a further reduction observed when *T. deion* was added (96.7%). There was a significant difference in live *P. interpunctella* among the treatments with bagged cornmeal alone, and *T. deion* on unbagged cornmeal, but not for the treatments with *H. hebetor* on bagged versus unbagged cornmeal (Fig. 2). Thus, the addition of *T. deion* to bagged cornmeal with *H. hebetor* present resulted in an additional reduction of 26.1% (Fig. 2).

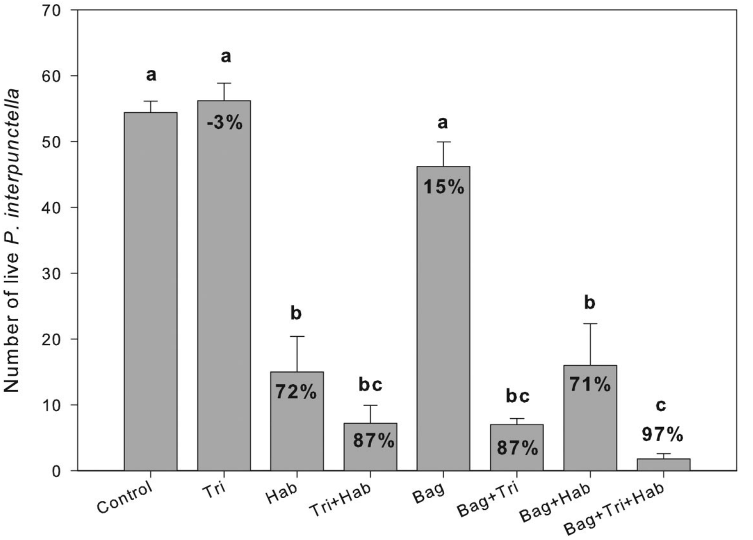

Fig. 2. Mean ± SEM total number of live *P. interpunctella*. Percentage of reductions compared with control are shown inside bars. Control, without bag, without *T. deion*, without *H. hebetor*; Tri, without bag, with *T. deion*, without *H. hebetor*; Hab, without bag, without *T. deion*, with *H. hebetor*; Tri + Hab, without bag, with *T. deion*, with *H. hebetor*; Bag, with bag, without *T. deion*, without *H. hebetor*; Bag + Tri, with bag, with *T. deion*, without *H. hebetor*; Bag + Hab, with bag, without *T. deion*, with *H. hebetor*; Bag + Tri + Hab, with bag, with *T. deion*, with *H. hebetor*. Different letters denote statistically significant differences (Fisher LSD; $P = 0.05$).

Discussion

Both *T. deion* and *H. hebetor* were capable of suppressing *P. interpunctella* population growth; however, the effectiveness of each species depended on whether the cornmeal was bagged or unbagged. *H. hebetor* played a major role in suppressing *P. interpunctella* larvae both in bagged and unbagged cornmeal. That *H. hebetor* contributed additional pest suppression on bagged cornmeal indicates that adult females are able to enter finely perforated packages and attack pests after infestation has occurred. In contrast, *T. deion* attacked *P. interpunctella* eggs on the surface of bagged cornmeal (preinfestation) and had a relatively greater impact on the pest population for bagged cornmeal than did *H. hebetor.* However, *T. deion* had no impact on *P. interpunctella* eggs when they were deposited in unbagged cornmeal. The likely reason for the large difference in efficacy of *T. deion* under the two conditions is that unbagged cornmeal resulted in a level of habitat complexity that interfered with the foraging behavior of *Trichogramma* females. Female wasps were apparently able to find and parasitize eggs much more efficiently on the smooth plastic surface of the bagged cornmeal.

Other authors have documented the detrimental effects of habitat complexity on host foraging by *Trichogramma* species. Andow and Prokrym (1990) demonstrated both a lower rate of parasitism and a reduction in giving up time on more complex wax and paper models, compared with simpler models. Additional laboratory studies by Lukianchuck and Smith (1997), Gingras et al. (2002), Gingras and Boivin (2002), and Andow and Olson (2003) have all shown similar results with less parasitism by various *Trichogramma* spp. observed in more complex arenas compared with simpler arenas. In the area of stored product protection, a study examining the foraging success of *Trichogramma evanescens* Westwood showed that *Trichogramma* were unable to locate sentinel egg cards that were covered with >2 cm of stored wheat (*Triticum* spp.) (Schöller et al. 1996). Furthermore, Grieshop (2005) demonstrated that the presence of millet or flour reduced the host-foraging success of three species of *Trichogramma.*

P. interpunctella suppression in cornmeal by *H. hebetor* did not seem to be affected by the presence or absence of bagging. Eight of 10 replicates with bagging and *H. hebetor* had the original adult and/or subsequent generations of adult and pupal wasps within the bagged cornmeal, indicating that bagging did not interfere with *H. hebetor* entering the bag and parasitizing *P. interpunctella* larvae. Similarly, unbagged cornmeal did not seem to hide hosts from *H. hebetor*, as evidenced by the large numbers of host larval corpses found within the cornmeal (Figs. 1 and 2). The difference in pest suppression between the two parasitoid species on unbagged cornmeal may be related to the larger size of *H. hebetor* or its use of larval kairomones as foraging cues (Strand et al. 1989), whereas *Trichogramma* rely on less concentrated chemical cues such as adult sex pheromones, wing scales, or egg volatiles to locate host eggs (Nordlund et al. 1981).

The differences in how *T. deion* and *H. hebetor* forage, combined with the presence of *P. interpunctella* in a variety of microhabitats in retail and warehouse settings, suggest that the greatest level of biological control could be achieved by releasing both parasitoid species. For example, *T. deion* should be able to locate host eggs on the surface of packaging, thereby protecting packages from infestation. However, Indianmeal moth eggs located in spillage or inside packages will likely prove to be inaccessible to *T. deion.* Furthermore, the preventive advantage of preinfestation mortality from egg parasitism by *T. deion* would probably be of little use in packages that have already been infested, both because *T. deion* seems to be ineffective in this situation and because any postegg life stages present would result in the total loss of the infested product (Subramanyam et al. 2001). However, *H. hebetor* seems to be well suited to foraging in bulk products or spillage, and it also may be capable of locating hosts within damaged packages. Therefore, in cases where *P. interpunctella* infestations are present in spillage or within packaging, *H. hebetor* may be useful by parasitizing host larvae, thereby reducing the next generation of adult moths or reducing the secondary infestation of packaged products by wandering larvae.

A larger scale study examining the use of *T. pretiosum* and *H. hebetor* for the suppression of the almond moth in simulated peanut warehouses also demonstrated that the combination of the two parasitoids prevented the most damage, compared with nontreatment controls and single-species parasitoid releases (Brower 1990). In addition, a study of *H. hebetor* as a preventative treatment to protect packages from infestation by the wandering instar of *C. cautella* demonstrated that *H. hebetor* was capable of protecting paper packages from invasion by moth larvae (Cline and Press 1990).

In conclusion, *T. deion* seems to be well suited to protecting packaged stored products from infestation; however, this species will probably be ineffective in areas with spilled products, which frequently occur under kick plates of shelving units. *H. hebetor* seems to be well suited to finding *P. interpunctella* larvae in spillage and perhaps even in damaged packages. Therefore, the combination of releasing both *H. hebetor* and *T. deion* should provide the best control when considered over both the short and long term. However, it is important to realize that unlike pest mortality resulting from egg parasitism by *T. deion,* larval mortality caused by *H. hebetor* will only prevent future infestation and does not protect packages from infestation by the current generation of *P. interpunctella.* Therefore, for adequate suppression of *P. interpunctella* to occur, releases should probably be made as early as possible, so that the parasitoids have a better chance of controlling pest populations before they reach economic levels.

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